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REGULAR ARTICLE

Novel $P2Y_{12}$ adenosine diphosphate receptor antagonists for inhibition of platelet aggregation (II): Pharmacodynamic and pharmacokinetic characterization

Joseph M. Post, Serene Alexander, Yi-Xin Wang*, Jon Vincelette, Ron Vergona, Lorraine Kent, Judi Bryant, Mark E. Sullivan, William P. Dole, John Morser, Babu Subramanyam

Berlex Biosciences, Richmond, CA 94804

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Abstract

Antiplatelet drugs are used to prevent aberrant platelet activation in pathophysiologic conditions such as myocardial infarction and ischemic stroke. The key role that ADP plays in this process has led to the development of antiplatelet drugs that target the $P2Y_{12}$ receptor. The aim of this study was to characterize the pharmacodynamic (PD) and pharmacokinetic (PK) properties of the novel $P2Y_{12}$ receptor antagonists, BX 667 and BX 048. BX 667 blocks ADP-induced platelet aggregation in human, dog and rat blood (IC_{50}=97, 317 and 3000 nM respectively). BX 667 had nominal effects on collagen-induced aggregation and weakly inhibited arachidonic acid-induced aggregation. BX 667 has an active metabolite, BX 048, that also potently inhibits ADP-induced aggregation (IC_{50}=290 nM) in human blood. BX 667 was shown to have high oral bioavailability in both dog and rat unlike BX 048. Administration of BX 667 resulted in a rapid and sustained inhibition of platelet aggregation where the extent and duration of platelet inhibition was directly proportional to circulating plasma levels. This report describes the PK/PD properties of BX 667 showing that it has the properties required for a potential antiplatelet therapeutic agent.

Introduction

Adenosine-5'-diphospate (ADP) activates platelets and is known to play an important role in haemostasis

* Corresponding author. Arete Therapeutics, Inc., 3912 Trust Way, Hayward, CA 94545. Tel.: +1 510 300 1868; fax: +1 510 785 7061.
E-mail address: yxwang2000@gmail.com (Y.-X. Wang).

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and thrombosis [1,2] through interacting with $P2Y_1$ and $P2Y_{12}$ [3–7]. Recognition of the therapeutic benefits of preventing thrombotic complications have led to the development of $P2Y_{12}$ receptor antagonists such as ticlopidine and clopidogrel which are used both for prevention and treatment of cardiovascular diseases such as atherosclerosis, myocardial infarction and cerebral vascular diseases [8–11].

Clopidogrel is an orally available irreversible $P2Y_{12}$ platelet antagonist that has been reported to be safer than ticlopidine [8]. Although, clopidogrel is an effective antithrombotic, it requires hepatic activation which delays inhibition of platelet aggregation and its irreversible binding to $P2Y_{12}$ receptors may prolong or exacerbate bleeding [8,12–14].

We have focused our efforts on the discovery and development of a $P2Y_{12}$ receptor antagonist with anti-platelet properties that will provide an improved alternative to currently available $P2Y_{12}$ receptor inhibitors. These efforts led to the discovery of the selective $P2Y_{12}$ receptor antagonists, BX 667 and BX 048. In part I of the companion paper, we describe the molecular pharmacology of these compounds. BX 667 is a potent and orally active $P2Y_{12}$ receptor antagonist which unlike clopidogrel, does not require hepatic activation and is reversible. In this paper we determine the pharmacokinetic (PK) and pharmacodynamic (PD) properties of BX 667 and BX 048. Metabolism of BX 667 results in the transition of the parent ester to its corresponding acid, BX 048, which is also a potent antagonist of $P2Y_{12}$ receptors. The *in vivo* activity of BX 667 could be explained by the sum of the PD activity of the parental compound and its active metabolite.

Methods

Chemicals

BX 667, "(S)-4-({4-[1-(Ethoxycarbonyl)-1-methylethoxy]-7-methyl-2-quinolyl} Carbamoyl)-5-[4-(ethoxycarbonyl) piperazin-1-yl]-5-oxopentanoic acid", and BX 048, "(S)-4-({[4-(1-Carboxy-1-methylethoxy)-7-methylquinolin-2-yl]carbonyl} amino)-5-[4-(ethoxycarbonyl) piperazin-1-yl]-5-oxopentanoic acid", were used in this study. The structures of the 2 compounds were published in the previous paper [14].

Whole-blood platelet aggregation

Platelet aggregation was assessed in heparinized (10 U/mL) blood diluted 1:1 with 0.9% saline induced with either 5 μM ADP, 3 μg/mL collagen (Type 1, Chrono-Log Corp, Havertown PA) or 300 μM arachidonic acid and incubated at 37 °C. Experiments were performed using human, male beagle dog or male Sprague-Dawley rat blood. BX 667 or BX 048 was added to individual cuvettes at half-log concentrations ranging from 0.01 to 30 μM. The cuvettes were then transferred to a whole-blood platelet aggregometer (ChronoLog, Havertown, PA), which measures impedance to quantify platelet aggregation. BX 667 or BX 048 were added 4 minutes prior to addition of agonist. Previous experiments in our laboratory have shown that use of these concentrations of ADP, collagen, and arachidonic acid produce a near maximal ($\approx IC_{90}$) aggregatory response by experiments in which whole blood was challenged with different concentrations of the agonists, platelet aggregation determined, and the concentration necessary for a 90% aggregation response was determined. The extent of platelet aggregation was calculated by measuring the area under the curve (AUC) for 15 minutes in the presence or absence of drug. Inhibition of platelet aggregation was expressed as a % inhibition with respect to controls (i.e. no drug added). IC_{50} values were calculated by averaging the concentrations from individual experiments necessary to inhibit agonist-induced responses by 50%.

In vitro stability and metabolism

Due to species differences in systemic esterase activity, the stability of the parent ester, BX 667, was evaluated in whole blood, hepatic microsomes and intestinal (S9) fractions of human, rat and dog. For evaluation in blood, BX 667 was incubated in heparinized whole blood at concentrations of 10 or 25 μM at 37 °C. A 0.5 mL aliquot was removed at 0, 15 and 30 minutes post incubation and plasma was immediately prepared. The resulting samples were denatured by addition of cold acetonitrile or methanol (1:4) containing an internal reference standard for quantification. The denatured proteins were removed by centrifugation and BX 667 was quantified utilizing LC/MS-MS.

The hepatic microsomal stability studies were conducted utilizing commercially available microsomes (Gentest Corp. Woburn, MA). Rat, dog and human hepatic microsomes of known P450 content and protein concentration were incubated with BX 667 and BX 048 at 37 °C at a substrate concentration of 10 μM. Incubations were carried out in the presence and absence of a NADPH generating cofactor system (8 mM glucose-6-phosphate, 4 mM $MgCl_2$, 1 IU/mL glucose-6-phosphage dehydrogenase, 0.5 mM $NADP^+$ in 100 mM sodium phosphate buffer). Following a specified incubation period, samples were quenched with ice cold methanol, precipitated proteins were removed by centrifugation and the remaining drug concentrations were determined by LC/MS/MS.

Testing of BX 667 hydrolysis to BX 048 in intestinal jejunal S9 fractions from human, dog and rat was performed by Quintiles (Kansas City, MO). BX 667 was incubated in triplicate with rat, dog and human jejunal S9, respectively, for 0, 5, 15 and 30 minutes at concentrations of 1 or 10 μM. Following incubation, acetonitrile containing 0.5 μM of an internal standard was added to terminate the reactions. Incubations were performed at 37 °C, in a 96-well plate on a Hamilton Microlab 2200 Liquid Handler. BX 667 and BX 048 concentrations were determined using ESI LC/MS.

Animal studies

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH).

For dog studies, male beagles were fasted for 18 hours and instrumented with indwelling jugular catheters on the day of the experiment for taking blood samples. Dogs were allowed water ad libitum and were housed individually. BX 667 or BX 048 was administered via oral gavage (PO) in a vehicle consisting of 100% propylene glycol or in gelatin capsules as a solid dose form or intravenously (IV).